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Evaluation of the Structure and Biological Activities of Condensed Tannins from *Acanthus ilicifolius* Linn and Their Effect on Fresh-Cut Fuji Apples

Chen-Fang Gong[1] · Yu-Xia Wang[1] · Meng-Li Wang[1] · Wei-Chao Su[1] · Qin Wang[1] · Qing-Xi Chen[1] · Yan Shi[1]

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Abstract

Condensed tannins (CT_S) have been isolated and purified from leaves *of Acanthus ilicifolius* Linn. And their structures were investigated by three methods: ^{13}C nuclear magnetic resonance (^{13}C NMR), reversed-phase high-performance liquid chromatography (RP-HPLC), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The results showed that the CT_S were a mixture of catechin/epicatechin, galatechin/epicatechin, and amphicin/epigalin, and that the polymer chain lengths were 3-mers to 14-mers. Antityrosinase activities and antioxidant activities of the CT_S from *A. ilicifolius* leaves were further studied. The IC_{50} of the CT_S on mushroom tyrosinase activity was determined to be 19.7 ± 0.13 μg/mL, and inhibition type analyses indicated that the CT_S were mixed type inhibitors and their inhibition CT_S was reversible. The CT_S from *A. ilicifolius* leaves also exhibited potential antioxidant activity. The IC_{50} of DPPH and ABTS scavenging activities were 104 ± 0.894 μg/mL and 86 ± 0.616 μg/mL, respectively. And the FRAP value was 758.28 ± 2.42 mg AAE/g. In addition, we found that the CT_S from *A. ilicifolius* leaves had an excellent effect on preserving the quality of fresh-cut apples by preventing apples from browning through reducing polyphenol oxidase activities in apples.

Keywords A. ilicifolius · Condensed tannins · Antityrosinase activity · Antioxidant activity · Preservation

Chen-Fang Gong and Yu-Xia Wang contributed equally to this work.

✉ Yan Shi
yshi@xmu.edu.cn

[1] School of Life Sciences, Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, Xiamen University, Xiamen 361002, China

Introduction

Acanthus ilicifolius Linn. (Acanthaceae), a molecular-marker-based mangrove species, is widely distributed [1]. The research on the chemical constituents and pharmacological activities of *A. ilicifolius* could be dated back to the 1970s [2, 3]. Previous studies have demonstrated that the root of the *A. ilicifolius* is an effective medicinal herb [4], but there are few reports on the use of *A. ilicifolius* leaves, and the research about the condensed tannins (CT_S) of *A. ilicifolius* leaves is still lacking.

CT_S are complex metabolic products in plants. They have rich chemical properties and different functions, which mainly depend on their structures. CT_S, also known as proanthocyanidins, are a kind of oligomeric and polymeric flavan-3-ols. The monomers of CT_S (Table 1**)** are linked through B-type linkage of C4-C6 or C4-C8, and A-type linkage of C4-C8 and an ether bond between O7-C2 [5] (Fig. 1). Therefore, the isolation and purification of the CT_S and their structural determination are challenging [6]. Three techniques have been applied to characterize the CT_S structures: RP-HPLC, ^{13}C NMR, and MALDI-TOF MS [7]. RP-HPLC is suitable for low degree of polymerization, and therefore, can be used to separate and analyze the CT_S with similar polymerization degree [8, 9]. ^{13}C NMR is mainly based on the displacement of the carbon atoms in the compound [10] and has been extensively used in the analysis and identification of the CT_S. It enables us to obtain the proportion of the propelargonidin (PP), procyanidin (PC), and prodelphinidins (PD), and the connection mode between each structural unit and the average degree of polymerization of the CT_S. ^{13}C NMR also has some drawbacks such as the extremely high requirement of the size, and purity of the sample MALDI-TOF MS has linear mode and reflection mode, and the reflection mode has higher resolution than linear mode. MALDI-TOF MS can be used to obtain the basic structure, proportion of the CT_S, degree of polymerization, and distribution range [11, 12].

Tyrosinase (EC 1.14.18.1), also called polyphenol oxidase (PPO), is a binuclear-copper enzyme. It is widely found in microorganisms, plants, and animals including human [13]. It is a crucial enzyme in relation to the enzymatic browning in fruits and vegetables. Some undesirable changes in color, flavor, and nutritive value of fruits and vegetables may be caused by tyrosinase [14]. It is also the key enzyme in the molting process of insects [15] and the formation of brown pigments [16, 17]. Importantly, in human melanocytes, abnormal tyrosinase expression can cause hyperpigmentation disorders, such as chloasma, black spot, freckles, and sites of actinic damage [18]. Although many synthetic tyrosinase inhibitors have been reported, the application is restricted due to their high toxicity and insolubility [19]. Therefore, the researches on how to apply the tyrosinase inhibitors from natural products in the treatment of dermatological disorders [20] and employ them as the key constituents of anti-browning agents [21] are extremely important.

Reactive oxygen species (ROS) in the human body are in a constant cycle of production and elimination. Excessive generation of ROS can cause a series of damages to the organism, such as heart disease, neurological disease, cancer, Alzheimer's disease, and atherosclerosis

Table 1 Common representation of condensed tannin structures

R1	R2	Monomer	Proanthocyanidins
H	H	Afzelechin/epiafzelechin (AF/EAF)	Propelargonidin (PP)
OH	H	Catechin/epicatechin (C/EC)	Procyanidin (PC)
OH	OH	Gallocatechin/epigallocatechin (GC/EGC)	Prodelphinidins (PD)

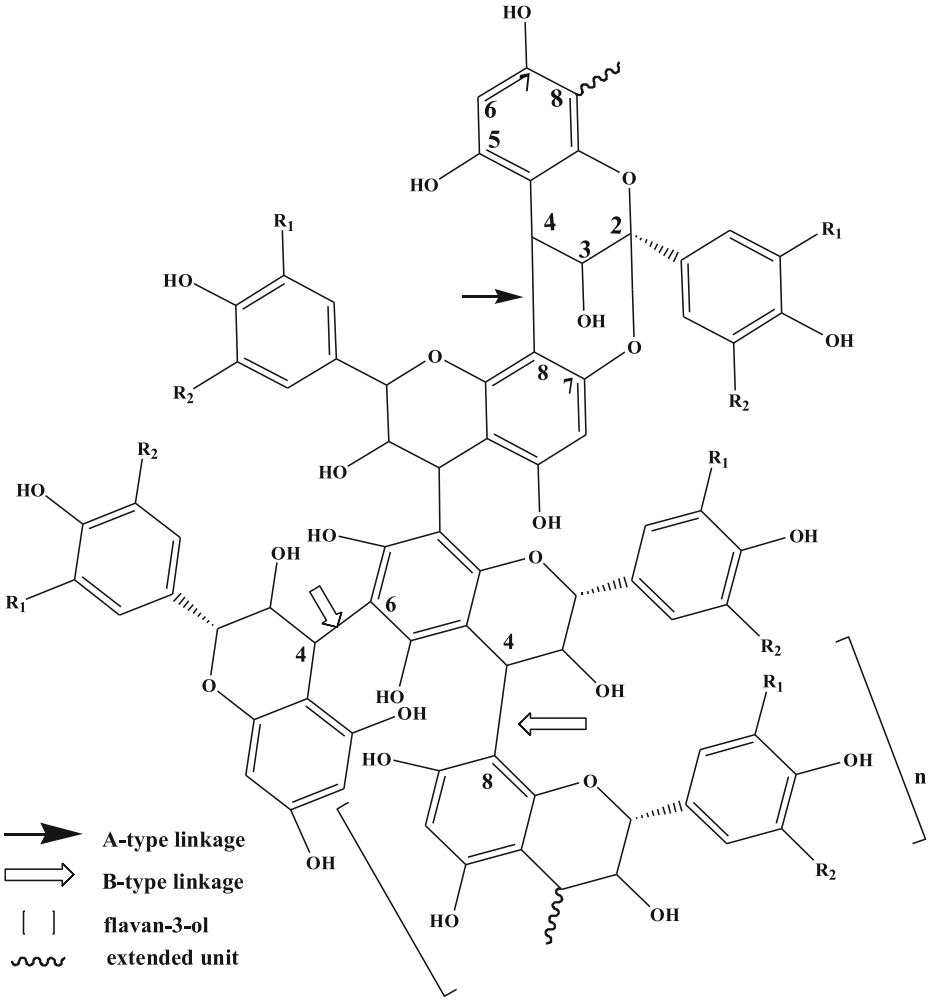

Fig. 1 Chemical structure of CT_S

[22, 23]. However, the potent scavenger of these ROS may serve as a possible preventive intervention of these diseases [23]. There are several reports that condensed tannins have good antioxidant properties [24–26].

Fresh-cut fruits are gaining popularity with modern consumers because of their convenience, and the demand for fresh-cut fruits in the market is increasing. However, fresh-cut fruits, such as apple slices, are prone to browning, softening, and microbial contamination due to the tissue damage caused by peeling and slicing, which will significantly lower their quality and reduce their shelf-life. Browning is a major concern related to the extension of shelf-life of fresh-cut fruit, and strongly affects the consumer's purchase decision. Browning is majorly caused by enzymes, such as PPO [27]. Traditionally, sulfites have been used for browning prevention. However, their use on fresh-cut fruit and vegetables was banned in 1986 by the FDA owing to their potential hazards to health [28]. Thus, much research needs to be done in order to find new techniques to prevent browning and preserve fresh-cut fruit products with high sensory quality and nutritional value.

With the primary goal of better application of CTs from *A. ilicifolius* leaves, we purified the CTs from *A. ilicifolius* leaves and determinated the degree of polymerization, the basic structure composition, and the proportion of the CTs. We also investigated the tyrosinase inhibitory activity and antioxidant activity of the CTs, and further examined their preservative effect on fresh-cut apple because of their potential anti-browning ability.

Materials and Methods

Sample Preparation

The *A. ilicifolius* leaves were gathered from Zhangzhou mangrove forest in Fujian, China. The leaves were freeze-dried, grinded to fine powders using a grinding mill (BL301D5, Saikang, China), and then stored at − 80 °C until analysis.

Chemicals

Mushroom tyrosinase (EC 1.14.18.1), L-DOPA, DMSO-d6, Sephadex LH-20, HPLC standards (catechin and epicatechin), amberlite IRP-64 cation-exchange resin, CsCl, benzyl mercaptan, DHB, ascorbic acid, TPTZ, DPPH, and ABTS were obtained from Sigma-Aldrich (USA). HPLC-grade CH_3CN and TFA were purchased from Sinopharm (Shanghai, China). The water used was double-distilled. Other reagents were of standard analytical grade.

Methods

Extraction and Purification of the CT_S

The method was according to Feng et al. [29]. Leaf powders (20 g) were ultrasonically extracted 3 times with 150 mL of acetone-water (70%, *v*/*v*) at 25 °C. Acetone was removed by rotary evaporation under vacuum at 40 °C. The remaining aqueous solution was extracted by 3 times with 150 ml ethyl acetate to remove low molecular phenolics and petroleum ether to remove lipophilic compounds and chlorophyll. The crude extract solution was concentrated by freeze-drying and separated by column chromatography (LH-20), which was first eluted with methanol-water (50%, *v*/*v*) to remove sugars, glycosides, and monomeric polyphenols and then eluted with acetone-water (70%, *v*/*v*). The fractions of 70% acetone-water were retained. The organic solvents were removed, and the aqueous fractions were freeze-dried to obtain purified CT_S powders. The powders were stored at − 20 °C before structure and activity analysis. The CT_S contents of *A. ilicifolius* leaves were 34.3 ± 2.24 mg/g.

^{13}C NMR Analysis

The Varian Mercury-600 spectrometer (Palo Alto, CA, USA) was used to record the ^{13}C NMR spectra of CT_S at a frequency of 150 MHz [12]. The CT_S were dissolved in DMSO-d6.

Reversed-Phase High-Performance Liquid Chromatography Analysis

The solution of the CT_S (0.5 g, dissolved in methanol), hydrochloric acid-methanol solution (3.3%), and benzyl mercaptan-methanol solution (5%) was mixed and incubated at 40 °C for 30 min. The mixture was then filtered through a filter (0.22 μm). The HPLC used for the CT_S was an Agilent 1100 system (Agilent, Santa Clara, CA, USA), equipped with a quaternary pump and a diode array detector. The column employed in the analysis was a 250 mm × 4.6 mm i.d., 5 μm, LiChrospher 100 RP-18, with a 4 mm × 4 mm i.d. guard column of the same material (Elite, Dalian, China). Catechin and epicatechin were used as standards. The solvents were 0.5% TFA and CH_3CN. The linear gradient elution system was 0–45 min, 12–80% CH_3CN; 45–50 min, 80–12% CH_3CN. The temperature of the column was 25 °C and the flow rate was 1 mL/min. The RP-HPLC analysis was carried out with reference to the previous report [30].

MALDI-TOF MS Analysis

MALDI-TOF MS analysis of the CT was performed using a Bruker Reflex III instrument (Germany). The reaction was proceeded at 337 nm wavelength, 3 ns duration, 20.0 Kv accelerating voltage, and 23.0 Kv reflectron voltage [31]. The sample solutions were mixed with CsCl solution at 1:1 (*v*: *v*). The mixture was mixed with the DHB matrix solution at 1:3 (*v*: *v*), and then spotted (1 μL) to the steel target.

Tyrosinase Inhibitory Activities

L-DOPA (0.5 mM) was used as a substrate in this experiment; the reaction medium was 3 mL and the final concentration of mushroom tyrosinase was 3.33 μg/mL [32]. The purified CT_S served as the effector. The enzyme activity was recorded at 475 nm by Thermo Scientific Multiskan Go. The inhibitory effect of the CT_S was expressed as the concentration that giving 50% inhibition of tyrosinase activity (IC_{50}). The inhibition type of the CT_S was determined by using Lineweaver–Burk plots, and the constants were obtained from the slope or the vertical intercept versus the CT_S concentration, respectively.

Scavenging Activity on DPPH Radical

The ability to scavenge DPPH of the CT_S was assayed according to Chen et al. [33] and was analyzed according to the formulae: DPPH % inhibition = $[(A_0 - A_1)/A_0] \times 100\%$. 0.1 mL of sample solution at different concentrations (12.5–200 μg/mL) was mixed with 3 mL of methanolic solution containing DPPH (25 mg/L). After the mixture was shaken vigorously and incubated in the dark place (25 °C, 30 min), the absorbance at 517 nm was read (A_1).

Scavenging Activity on ABTS Radical

The ability of the CT_S to scavenge the ABTS radical was evaluated by the method with some modifications [34]. $ABTS^+$ working solution should be prepared in advance. Firstly, the ABTS (7 mM) and potassium persulfate (2.45 mM) were mixed in the dark place for 16 h. Then, it was diluted with 80% alcohol solution, to achieve the absorption value 0.700 ± 0.05 at 734 nm. After that, 0.1 mL of sample solution at different concentrations (12.5–200 μg/mL) was mixed

with 3.9 mL of $ABTS^+$ working solution. The mixture was incubated at 25 °C for 6 min and the absorbance at 734 nm was recorded. An equal amount of 80% ethanol served as the control. The equation is the same as the DPPH radical scavenging activity assay.

Ferric Reducing Antioxidant Power

The FRAP assay was carried out according to the method established by Benzie and Strain [35]. The freshly prepared FRAP reagent, TPTZ (10 mM), ferric chloride (20 mM), and sodium acetate buffer (pH 3.6) were mixed at the ratio of 1:1:10. Then, 0.1 mL of the sample solution was mixed with 3 mL of FRAP reagent. The mixture was incubated 10 min at 25 °C and the absorbance was determined at 593 nm.

Fruits Treatments

Fuji apples (*Malus domestica* Borkh. cv. Red Fuji) were purchased from a market at Xiamen, China, and selected for uniformity of shape and maturity. The apples were pre-cooled at 4 °C overnight before they were peeled and sliced into uniform pieces. Experiments selected 0.5, 1, 2, or 4 mg L^{-1} CT_S as sample solutions. The apple slices were dipped immediately in distilled water (control) or four CT_S solutions at different concentrations at room temperature for 15 min. To drain excess solution, all treated apple slices were placed on a plastic sieve for 15 min and then packed into polyethylene bags and kept in 4 °C refrigerator until further analysis.

Measurement of Storage Quality

During storage, the weight loss, surface color (L* value), ascorbic acid content (Vc), and PPO activities were measured at an interval of 2 days in triplicate to evaluate the storage quality of fresh-cut apples. To determine weight loss, duplicate samples were stored separately. The total weight loss of fresh-cut apples was calculated on initial weight basis and expressed in percentage. L* value change of fresh-cut apples was determined using an ADCI-60-C colorimeter according to the method designed by Hu et al. [36]. Ascorbic acid was extracted and assayed by the reported method [37], the results were expressed on a fresh weight basis as μg/g. PPO activities were determined according to the previous research [38], the result was expressed as units of enzyme/mg protein.

Results and Discussion

^{13}C NMR Analysis of CT_S

The liquid-state ^{13}C NMR spectrum was observed in a DMSO-d6 solvent and was presented in Fig. 2, in which, different signals were assigned according to previous studies [39, 40]. The presence of propelargonidin (PP) together with procyanidin (PC) and prodelphinidin (PD) could be found in the ^{13}C NMR spectrum. Especially, the stereochemistry of the C ring was sensitive in the region between 65 and 85 ppm. C2 gives 76 ppm and 83 ppm for the cis and trans forms, respectively. Both cis and trans forms of C3 occurs at 71 ppm. The presence of C8, C4a, and C6 of PC units, C6′ and C2′ of PD units can be obtained through the region

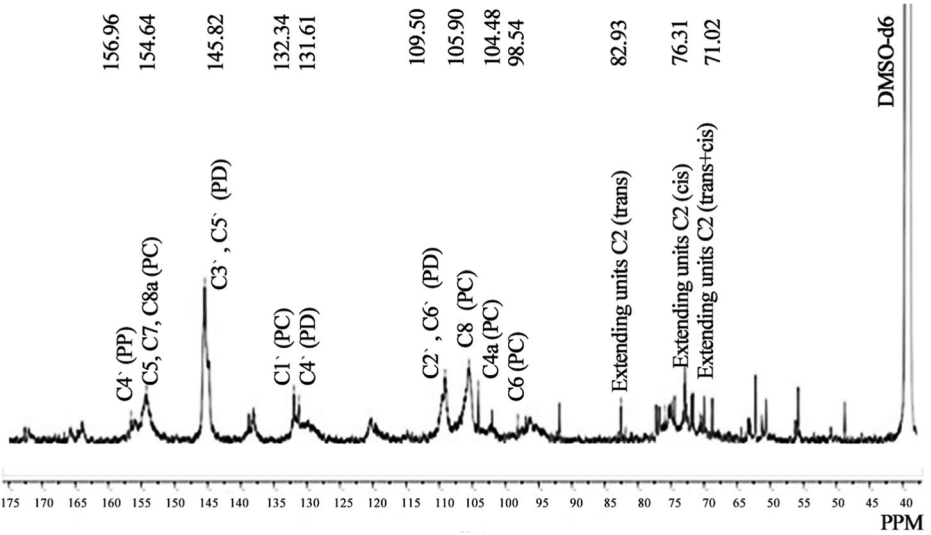

Fig. 2 ^{13}C NMR spectrum of the CT_S from *A. ilicifolius* leaves

between 110 and 90 ppm. The two peaks at 132 and 154.6 ppm appear corresponding to the C1′ and C5、C7、 and C8a of PC units. The signals at 109.5 and 131.6 are assignable to the C2′, C6′, and C4′ of PD units. Peaks at 145.8 ppm also belong to C3′ and C5′ of PD units. A small amount of PP (C4′) was also detected at 157 ppm.

RP-HPLC Analysis

RP-HPLC has the characteristics of high efficiency, high speed, and high sensitivity. CT_S ingredients can be qualitatively distinguished according to the molecules and ions produced. The identity of the individual units that make up a CT_S polymer and the length of the polymer can be estimated by subjecting the polymer to strong acid-catalyzed cleavage in the presence of benzyl mercaptan. These reactions result in the release of terminal units as free flavan-3-ols, whereas extending units are distinguished as benzylthioether adducts [41, 42]. In our previous reports, degradation products of thiolysis were analyzed by reversed-phase HPLC-ESI-MS, and the results were summarized for further studies [33, 43]. The chromatogram (Fig. 3) showed that the terminal units of the CT_S consisted of C/EC and GC. The extension units in CT_S contain C/EC-benzylthioether, GC/EGC-benzylthioether, and AF/EAF-benzylthioether, with the predominance of GC/EGC-thio. Therefore, the chemical composition of CT_S from *A. ilicifolius* leaves analyzed by RP-HPLC was consistent with the^{13}CNMR results. Following the results and our previously reports, the retention time (RT) for the standards and the CT_S samples from *A. ilicifolius* leaves was presented as Table 2.

MALDI-TOF MS Analysis

^{13}CNMR and RP-HPLC provide a general understanding of basic unit composition of the CT_S from *A. ilicifolius* leaves. To further analyze the high degree of polymerization, MALDI-TOF MS analysis was performed [44]. As Fig. 4 showed, the positive-ion MALDI-TOF MS of the CT_S, recorded as Cs^+ adducts in the reflection mode, showed repeating peaks; the masses of

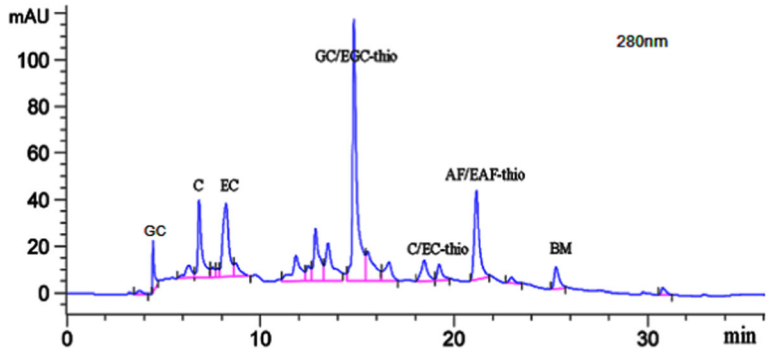

Fig. 3 RP-HPLC spectrum of the CT_S from *A. ilicifolius* leaves. Terminal units: C, EC, and GC. Extender units: GC-thio, EGC-thio, C-thio, EC-thio, AF-thio, and EAF-thio. BM, benzyl mercaptan

the highest peaks among the CT_S polymers with identical degree of polymerization increased at the different distance of 288 or 304 Da in leaves, corresponding to C/EC and GC/EGC monomers. It indicated that the main structural elements of the CT_S included C/EC and GC/EGC. The polymerization degree of the CT_S from *A. ilicifolius* leaves is from the trimer to the tetramer. When the heptamer spectrum was enlarged, a subset of peak with mass 152 Da and 162 Da lower than the highest peak was detected, with a distance of 152 Da corresponding to the addition of one galloyl group at the heterocyclic C-ring, and a distance of 162 Da corresponding to the addition of one glucose group at the heterocyclic C-ring. In addition, the subset of masses 14 Da higher was also detected, which can be explained by GC/EGC containing an additional hydroxyl group at the position R2 of the B-ring and the monomers are linked through A-type linkage.

Effects of CT_S from *A. ilicifolius* on Mushroom Tyrosinase Activity

The influence of the CT_S from *A. ilicifolius* on the kinetics of L-DOPA catalytic oxidation by tyrosinase was further evaluated and the inhibitory effect of the CT_S was determined. As Fig. 5 showed, the CT_S from *A.ilicifolius* leaves presented tyrosinase inhibitory activity with an IC_{50} value of 19.7 ± 0.13 μg/mL. Among commercial tyrosinase inhibitors, arbutin had received great attention as an effective mixed inhibitor of tyrosinase, the IC_{50} on mushroom tyrosinase was 10.89 mg/mL [45], which was remarkably larger than that of the CT_S Therefore, our study indicated that CT_S exhibited high potential mushroom tyrosinase inhibition activity.

Inhibition Kinetics of Mushroom Tyrosinase

The IC_{50} of the CT_S from *A. ilicifolius* leaves showed an effective inhibitory activity, which was further studied to determine the mechanism and type of inhibition. The inhibitory

Table 2 Retention time obtained by RP-HPLC

Flavan-3-ol		GC	C	EC	GC/EGC-thio	C/EC-thio	AF/EAF -thio	BM
RT (min)	Standards	4.4	7	8.5	15.4	18.5	21.5	27.5
	CT_S	4.3	7.1	8.4	15.6	18.7	21.5	25.6

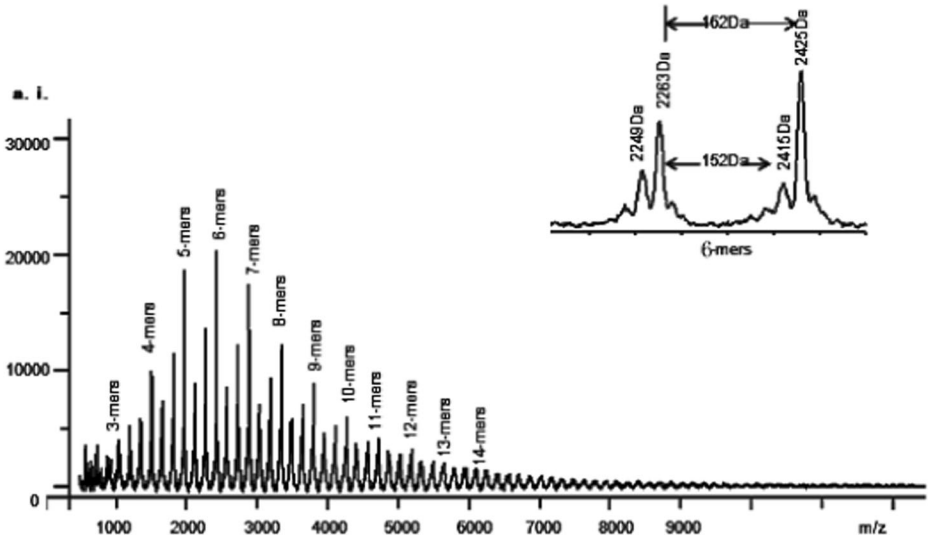

Fig. 4 MALDI-TOF MS spectrum of the CT_S from *A. ilicifolius* leaves

mechanism of the CT_S from *A. ilicifolius* leaves on tyrosinase was based on the relationship between the enzyme concentration and remaining enzyme activity. As Fig. 6a indicated, plots of the velocity versus the tyrosinase concentration presented a family of straight lines passing through the origin, and the slope decreased with increasing concentrations of the CT_S, indicating that the inhibition was irreversible. In addition, inhibition kinetics was analyzed by Lineweaver–Burk plot. When the enzyme concentration was fixed, and the concentration of L-DOPA was changed as Fig. 6b showed, the plots of $1/v$ versus 1/[S] presented a family of straight lines with different slopes and intercepts, which crossed at the second quadrant,

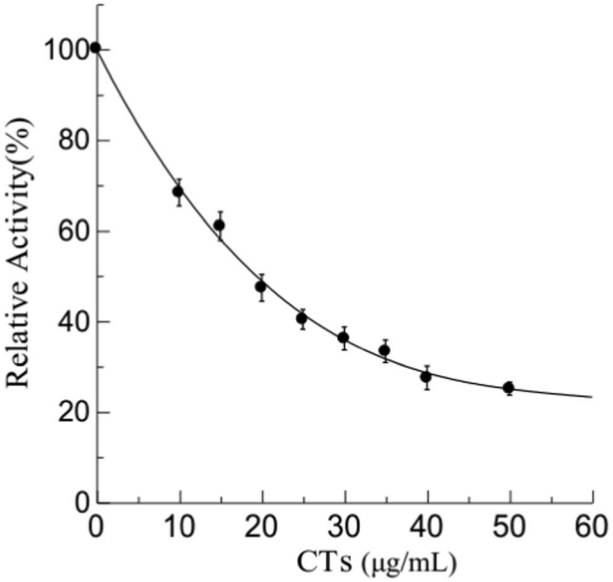

Fig. 5 Inhibitory effect of the CT_S from *A. ilicifolius* leaves on mushroom tyrosinase activity

indicating that the CT_S from *A. ilicifolius* leaves were a mixed type inhibitor. Plots of slopes and intercepts recovered from the Lineweaver–Burk plot regression calculations, and the inhibition constants (K_I, K_{IS}), were obtained by the secondary plots of the slopes or the vertical intercepts versus the concentrations of the CT_S (Fig. 6c, d). The values of K_I and K_{IS} were determined to be 17.19 μg/mL and 29.91 μg/mL. The value of K_I was lower than that of K_{IS}, indicating that the CT_S were more likely to bind the free enzyme than enzyme −substrate complexes.

Antioxidant Activity of the CT_S from *A. ilicifolius*

Antioxidant activities of the CT_S from *A. ilicifolius* were evaluated by DPPH, ABTS, and FRAP assays. These assays can better reflect comprehensive antioxidant effects [46]. The method of DPPH was used to evaluate the activity of a sample to scavenge DPPH free radical, and the color of the reaction corresponded to the decrease of the absorbance value [47]. ABTS method was adopted for the determination of the ability of a sample to scavenge the ABTS radical, while FRAP assay was used to measure the total antioxidant capacity, based on the ability of a sample to reduce iron under acidic conditions. All three methods were compared with a standard antioxidant, and ascorbic acid was used in this study. The antioxidant capacity of the CT_S was measured by the EC_{50} values, the concentration of 50% scavenging activity. A lower value of EC_{50} indicates greater radical scavenging activity and antioxidant activity. The antioxidant properties of the CT_S from *A. ilicifolius* leaves by three methods were presented in Fig. 7. The DPPH EC_{50} value and the ABTS EC_{50} value were 104 ± 0.894 μg/mL and 86 ±

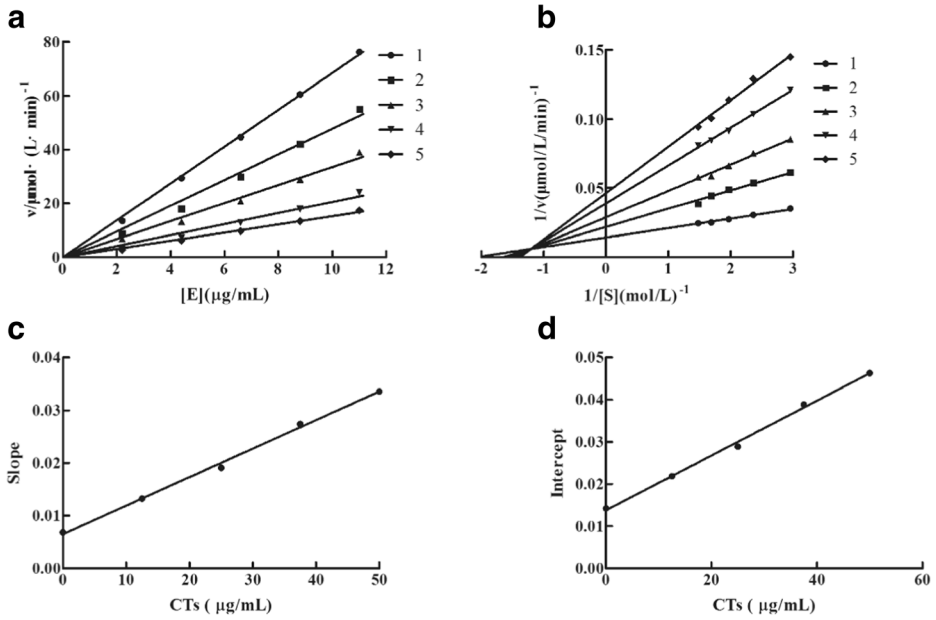

Fig. 6 Determination of the inhibitory mechanism (**a**). Lineweaver–Burk plots for mushroom tyrosinase with L-DOPA as substrate in the presence of the CT_S (**b**). Slopes and intercepts were used to calculate K_I (**c**) and K_{IS} (**d**) inhibition equilibrium constants, respectively. The concentrations of the CT_S for curves 1–5 were 0, 12.5, 25, 37.5, and 50 μg/mL, respectively

0.616 μg/mL, respectively, which were higher than that of vitamin C equivalent under the same conditions. The FRAP value of the CT_S from *A. ilicifolius*, which was expressed in ascorbic acid equivalents, was 758.28 ± 2.42 mg AAE/g, indicating its higher antioxidant potential (Table 3). The above experiments showed that the CT_S from *A. ilicifolius* leaves exhibited high antioxidant activities.

Evaluation of CT_S Treatment on Fresh-Cut Apples

The weight loss rate is used as the appraisement standard of fruit freshness. As shown in Fig. 8a, with the prolongation of storage time, the weight loss rate of apples increased correspondingly. The treatment with CT_S could reduce the weight loss of apple, and the reduction was significantly lower compared with the control group. After 8 days of storage, the weight loss of the apples in the control group was 80%, in contrast to only 21% and 38% in the group treated with 2 and 4 mg/mL CT_S.

The visual appearance of fresh fruits is one of the key determinants of quality to a buyer and color is one of the most important appearance attributes. As we know, the browning degree increases with prolonged storage time, whereas the L* value decreases [48]. And a higher L* value indicates a lighter surface and a slight browning [49]. In this study, the L* value showed a trend of continuous decline with the extension of storage time, and the change of L* value of the treatment with CT_S was slighter than the control group (Fig. 8b), indicating that the treatment with 2 mg/mL CT_S had the stronger effect on maintaining the color of fresh-cut apples.

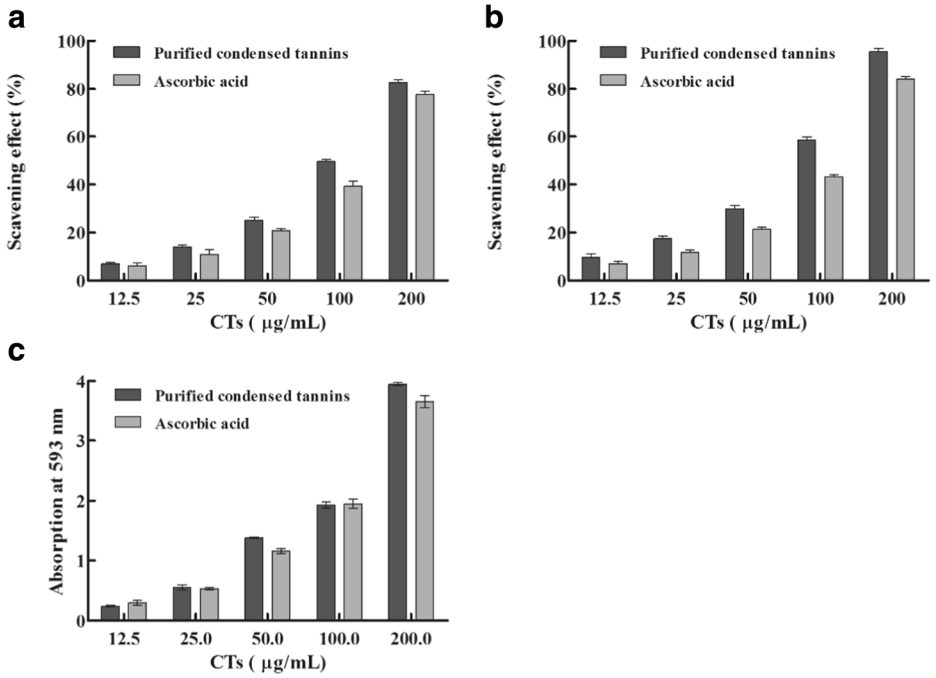

Fig. 7 Evaluation of antioxidant activities of the CT_S from *A. ilicifolius* leaves by DPPH (**a**), ABTS (**b**), and FRAP (**c**) methods

Table 3 Antioxidant activities of the CT_S from *A. ilicifolius* leaves

Sample	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	FRAP (mg AAE/g)
Leaf	104 ± 0.894	86 ± 0.616	758.28 ± 2.42
Ascorbic acid	131.47 ± 1.90	119.97 ± 0.463	–

Ascorbic acid, as an antioxidant compound, is a natural inhibitor of plant browning and can be effective in the prevention of enzymatic browning [50]. Therefore, the content of ascorbic acid is considered an important quality index of fruits. The effects of CTs at different concentrations on the content of ascorbic acid are illustrated in Fig. 8c. During the whole period of storage, the ascorbic acid content of apples showed a downward trend both in the control group and the CT_S-treated group, but the treatment group inhibited the downward tendency. After 8 days of storage, the ascorbic acid content of the 4 mg/mL CT_S-treated apples was 1.26 times greater than those in the control group.

Some enzymes were released during the storage of fresh-cut tissue, especially PPO, which can catalyze the oxidation of polyphenols in tissues and cause damages and browning [51]. Figure 8d showed the changing trend of the PPO activity during the storage. The PPO activity in the 0.5 mg/mL CT_S-treated apples just increased during the first 2 days of storage and then remained slight declines until the end of storage, while the PPO activity in the 1 and 2 mg/mL CT_S-treated apples increased on day 1–4 and decreased on day 6. At the end of storage, the activity of PPO in control slices was nearly 2 times higher than that of the 1 mg/mL CT_S-treated apples.

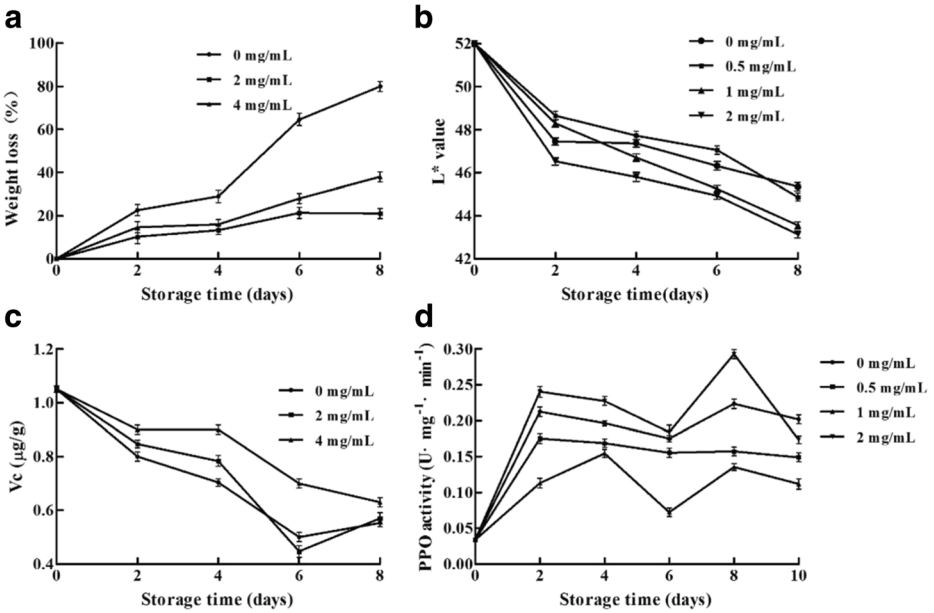

Fig. 8 Effects of the CT_S from *A. ilicifolius* leaves on the preservation of fresh-cut apples. Changes in weight loss (**a**), L* value (**b**), Vc content (**c**) and PPO activity (**d**) treated with different concentrations of CT_S

Conclusion

The CT_S are complex in origin and diverse in structure, making the purification and analysis of CT_S challenging. In this study, the structures of the CTs from *A. ilicifolius* leaves were firstly characterized by the methods of ^{13}C NMR, RP-HPLC, and MALDI-TOF MS. Through the methods of ^{13}C NMR and RP-HPLC, the basic structure of the CTs from *A. ilicifolius* leaves was roughly understood to possess AF/EAF, C/EC, and GC/EGC. The further characterization of the CT_S was studied by MALDI-TOF MS. In this method, the sample is surrounded by high concentration ionizing reagent Cs^+, which can guarantee the integrity of the sample and avoid sample fragmentation. It was found that the degree of polymerization of the CT_S was distributed from trimer to tetramer, some of the polymers also contained galacyl-gluconate substituent. Moreover, we found that the CTs from *A. ilicifolius* leaves have tyrosinase inhibition activities and are reversible inhibitors, which indicated that the effectors could decrease the activity of tyrosinase without the concomitant amount change of the efficient enzyme. Furthermore, the CT_S from *A. ilicifolius* leaves could inhibit both the free enzyme and enzyme–substrate complex. In addition, the antioxidant activity of CT_S from *A. ilicifolius* leaves was tested. Our results revealed that the CTs from *A. ilicifolius* leaves can be developed as a market-viable antioxidant. Furthermore, the effect of the CT_S from *A. ilicifolius* leaves on the preservation of fresh-cut apples suggested that it could effectively prevent browning and inhibit PPO activities. In conclusion, the CTs from the *A. ilicifolius* leaves can be developed as an antityrosinase and antioxidant with promising prospects in many fields, such as food preservation, nutraceutical, cosmetics, and medicine.

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Compliance with Ethical Standards

The authors declare that there are no competing financial interests.

Abbreviations

CT	condensed tannins
L-DOPA, L-3, 4	dihydroxyphenylalanine
DMSO	dimethylsulfoxide
DPPH	2,2-diphenyl-1 picrylhydrazyl
ABTS	2,2-azino-bis (3-ethylbenzothiazoline-6-sulfoni acid
CsCl	cesium chloride
DHB	2,5-dihydroxybenzoic acid
TFA	trifluoroacetic acid

TPTZ	2,4,6-tripyridyl-s-triazine
PPO	polyphenol oxidase

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